

October 7, 1951.

- A. For S^D/S^S Cross on EM5Lac, EM5Malsun.
- B. 10/8/51. H292 x W1709

No yield!!!

Check purity of W1709. Grown on Peucetia + 1000 usy/^{ml} (hi)

S^D (lo)

(10/11/51)			
A	1709^{Lo}	x	W1490
			High yield
B	"	x	H292
			ng - overgrown
C	1709^{Hi}	x	W1490
			V. High yield
D	1709^{Hi}	x	H292
			ng-
E	1709^{Hi}	x	58-161
			3-4
F	1709^{Lo}	x	58-161
			3

Note reduced yield of $S^D \times S^S$ cross. (Residual sun in S^D cells?). Should compare $S^S \times S^D$; S^R grown on comparable sun medium, and $S^S \times S^R$ on plain non-sun broth.

- su876A A W1734 x H290 Low yield on EM5Lac (ca 5%); ca 200/EM5Malsun.
- su877 B " " x H291 Very low yields ca 4/EM5Lac ± sun.

W1734 x H290
Mal + S^D Mal - S^D II

876 A

A. EMS Lac.
B. EMS Mal sm.

Stock out EMS Star, EM13 Mal, EM13 Mal sm
High yield, but n.g. 246.

	Mal	Mal sm.
1	v?	
2	v	
3	v	
4	?	
5	v	
6	v?	
7	v	
8	v	

growth
much
sparser
colonies
indistinct
May be ~~not~~ S^D S^D
with interbund S^D
segregants.

Presumably S^D dom S^D

Repeat 10/19/51. see 877.

10/21: ca 5-10 pupate Pick 1-4.

10/22 Pick 5-8. incl. 102 v. small Lg.?

	Lac	Mal	EMS Star sm.
1	+	nov	S
2	+	"	S
3	+	"	S
4	+	-	S
		not S v	

} No colonies
in aphids
to EM13 sm.
see 878.

Picks from
EM13 Mal.

	Lac	Mal	Lac sm. Mal sm.
5	++		- (few) Mal sm
6	++		- (few) Mal sm
7	++		" "
8	++		" "

Picks single colonies from EMS. Brush from EM13 Lac, Mal.

No Mal v.

11/2/51

S^DS^R Mal

C W1734 x H301 v
 D " H302 - No yield.
 Yields very low, improved over s.

Plate in EMS lac. Streak sas some plates to establish gradient of s concentration.

D. Experiments continued in desultory fashion.

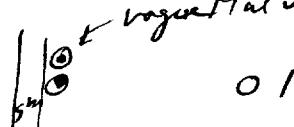
Many prototrophs fail to grow further. In numerous (20-40?) sets, lacv were mostly Mal-, some Mal+, 2-3 Malv but no S^D were noticed.

Investigate further S^D prototrophs. 10 reisolated

EMB/Mal

1	+D	-
2	+D	-
3	+D	-
4	-	
5	-	??
6	-	5 may be S ^D or S ^D /SR
7	-	
8	-	
9	+D	-
10	+D	-

3, 4, 7, 9, 10 did not produce good colonies on EMS lac sas (100 u/ml)

4 (single colony?) shows  0 Mal- 4D 1-3 s.c. most definite signs of being a heterozygote. Picks single "Malv" colonies 4D4 grown from EMS lac sas

5 shows strong Mal+ S^D reaction and a single to Mal- S^D.

Purity of initial strains is doubtful

	EMB/Mal 1/5m		EMB/lac 1/5m		EMB/lac sas		EMB/Mal sas	
D4:	1	+D	-D	v?	v-	-	+	v?
?	3	+D, "R	v?	v, -	(D?)	v-	-	v?
	4-mix	- R	v?	v, -		v-	+,-	v?

Picks clear single colonies, Malv or lacv and streaks EMB, EMS ± sas

W1734 x H291
Mal+S^D Mal-S^R

877

A EMS Lac
B " Malvin

A. EM8 Mal av. + son.

1	v	v
2	v	v
3	v	v
4	v	v
5	v	v
6	v	v
7	v	?
8	v	v

On EM8 Mal, a more or less "normal" segregation is observed, Mal+ predominant (not necessarily not v.)

With son, Mal- is much more prominent, suggesting that S^D/S^R in presence of son does not compete well with ~~Mal+~~ S^R.

If S^D is present here, it is presumably recessive to S^R.

B. Mal v very doubtful. same appearance ± son.

Lost by overheated incubator.

Repeat 10/21/51. ca 5-10 colonies per plate EMS Lac. Pick (1-4).

10/19/51. 10/22 Pick (5-8) including numerous small lac?

Lac Mal. EMS son

{ 4	nov.	+	nov?	+	S
2 +	"	+	"	+	S
3 +	"	+	"	+	S
4 +	+	##	"	+	S

Test colonies on EM8, EM8 ± son.
from EM8 by replica plate.

#39, occ. colonies from thick streak gave
Mal- colonies on EM8 malvin. Mostly Mal+S^R.

Picks doubtful colony from 1, 2, 3. 1a, 2a are Mal+

3a is Mal v. Mal- on EM8 Malvin. ~~probably S^D or S^R~~

Maybe either S^R/S^S or S^D/S^S, probably the former!

Not prototroph: probably H291 parent!

5
6
7
8

Lac only Mal on
" " "

all are S^R/S^S

Note H291 found to
be Mal v.

Preserve H298

818

4PM 10/16/51

A. Dilute 1:100 in

1. Water	a bc	= 1 - 3
2. Saline	a bc	= 4 - 6
3. Peptone 2%	a bc	= 7 - 9

Initial assay: lost

Probably ca 2×10^9

B. Broc .05 ml in
a Old Silver
b New "
c Gran "

C. New Silica, Peptone dil.

ca. 1.5 g silica per tube.

.01		10	0
.02		11	0
.05		12	0
.1		13	0
.2		14	5
.5		15	4
1.0	ml.	16	5×10^4
		Remained wet	

11/18/51. Nitrate assays. Diluted initial would be ca. $2 \times 10^7 \times \cancel{10} \times .05$ per
10 ml tube, = 10^5 /ml. Plate .1 ml and .001 ml samples.

#1, 2, 3, 5, 8 showed no survival in 1 ml samples. However, every tube grew out after 48 hours (cells bound to silica??)

∴ Water dil., or ~~or~~ saline opal, or any, or saline or peg in "new" slices
not very good.

15. 4 colonies at 10^{-1}

#16 5 colonies at 10^{-5} ! (but this not dried)

Many apparently "Lac+" colonies.

11/20/51 Plates of remaining tubes: 4, 6, 7, 9, 10, 11, 12, 13 sterile
(over) #14 shows 5 colonies at 10^{-1} turbid.

These results are very discouraging. However, they
may be the result of early destruction or a poor mixture
for the initial assay was lost in an unheated
incubator. of 872 B1.

Glu- x Gal- = Lac-?

10/20/51.

W990 = Y10 Glu-Lac+

W618 = 58-161 Gal-Lac+

Gal	Glu	W
+	+	1741
-	-	1742
+	+	1743
+	-	1744

Cross on EMS Gal. Replica to Gal, Lac, Nutt.

Pick the 4 combinations: all are Lac+!

In the cross, $\text{Gal}^+ \gg \text{Gal}^-$
 $144L^- \gg 144L^+$

suggesting that the Gal and Glu- are both linked to B11.

[W619 was also tested, but this is Lac±. The genetics of the loci here is not known. Should try W618 x W251, 252.]

On fermentation tubes, rather slow ambiguous reactions were seen.

W1742 was grown on Y+Lac plates, harvested to water and suspension tested for glycolysis in 1% buffer, 1% sugar BCP.
(15 minute test).

Lactose	++
Glucose	+++
Galactose	±

This suggests differential adaptation of glucosidase to lactose!
Compare glucose, lactose grown cells.

W251 (Lac+Glu-Gal+) x W618. Good yield
Isolate several Glu-Lac+ Gal-

W1752 1753.

W251 in fermentation tubes is Gal++ Glu+ Lac±! in
contrast to appearance on EMB plates.

SL 545

11/8/51.

Culture WAc 1 Received from E.S. McCoy. Transfers to Nutrient Agar slants, streaks out mother mold. Limited vegetative growth on EryBac, Glycerol. No growth on FTS. Good growth, limited sporulation, on D(C) agar. Culture inhibited by streaked loopful of sun 10^5 /ml.

11/14 Harvest spores from n.s. slant in 10 ml H₂O with vibrato, ca 10 minutes. Count ca 2×10^8 in counting chamber. Adjust to ca 10^8 /ml in H₂O. Many clumps; ca $\approx 80\%$ single spores. Dilute + plate out on nutrient agar.

A) control B) uv 60 sec. C) uv+120 sec.

Plate 10^6 , 10^5 , 10^4 A., B., C.

For irradiation, dilute ~~to~~ to 10^5 /ml predicted count.

A 6	<u>111</u>	B 6	<u>125</u>	replica to nutrient agar	No appr. kill at 60 sec.
↓		8 poss. streaked		transvibrato	
4 pos. tested, all +		Zuvotgraphs : WAc-2, -3	-	try 90 sec.	

11/16 Dilute to nominal 10^4 /ml

A 1 .05 ml

A 2 .02 ml

B 90 sec. \cdot^1 ml

C 120 " " 0

D 150 " " 0

E 180 " " 0

204
233
48

No outgraphs

6, 14, 4, 8, = 32 ~~aux.~~ no aux.

Survival.

11/18 F 90 sec, Dilute ~~to~~ to nominal 10^5 /ml .05 ml/plate
5 plates, ca 80 scoreable/plate (+ bacterial contaminants!)

2? aux. No.

(See over)

6 60sec ca 500 colonies 1 ?? WAc 4
Grows slowly, comparably on minimal agar.

Probably more slowly than WAc 2.

1 colony noted as producing pale yellow pigment on minimal agar.

I	60sec	8 plates	ca 90/	720
J	90sec.	12 "	ca 45/	540
-				1260 colonies.

10 possible mutants:

2 from J

8 " I (5 on 1 plate!)

Eventually 13 possible mutants.

4 show v. low residual growth on minimal agar
(1, 2, 6, 10) = WAc 5-8

Others are mixtures or slow types. Rechecks up to 50% from streak plates

WAc 3 x 4

12/20/51.

WAc 2	A1	Slow growth on minimal, vits?	Arginineless
3	A2		Lysineless.
4	A4		

WAc-2 grows more slowly than + but eventually (3-5 days) gives considerable growth. Combinations with WAc1 show no improvement, either by cross-brushing on minimal agar (D(0)) or by streaking from X on nutrient agar.

WAc	5	6	A1
	6	10	A2? Synt. WAc3
	7	1	A4
	8	2	A4?
	9		Slow growth.

• when 1st streaked on minimal agar
selected colonies with poor growth
in peripheral sectors

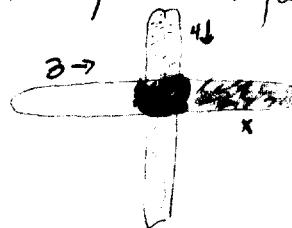
12/21/51 Cross brush suspensions of WAc 2, 3, 4 on minimal agar.

12/2/51. Controls: WAc 3 has a barely visible residual growth; 2, 4 show definite residue, (2 > 4).

2 x 3 Definite improvement (aerial mycelium) at cross-brush.

2 x 4 Marginal " " " " "

3 x 4 Heavy aerial mycelium at cross brush and at canyons regions:



Proper demonstration of interaction
may depend on using slightly
"negative" mutants.

12/6. Patches from x, cf. parent

- 4 A4 ✓ A1?
 5 A1 ✓
 6 V.t., A4, A1, A3, A5
 7 A4 A1? esp. A2.
 8 A4 (weak?)

anthers, reaction is graded with
++ toward old spot of WAc 3

From experiments on reactions on the vegetative
parts used in fig. 3

WAc 8 shows strong synthyphism
with 880-9 (WAc 9)

Cross brush var. comb WAc : large spread, but
apparent interactions are
 5 x 3 (maybe 5 x 6, actually)
 6 x 8 "
3 x 7 very clear.



WAc 8



WAc 3

Repeat on separate plates, 12/8/51.

3 x 5

6 x 8

5 x 6

12/10 12/12
 ++ (no spot reactions)
 ++ { streaks are in controls
 ++ } indefinite owing to synthyphism. All ++

11/20/51.

See 765

Original plants W1327-1330 were restudied for purposes of recovery and storage. All are predominantly stable +, -, or slow, no unstable forms seen at first sight. Mal- forms are apparently stable now! This is contrary to former result of high instability. Attempt repeated upatings!

W1729 was noticed as a similar Mal- type. Byophil culture predominantly Mal-. Following experiments are designed to ① establish patterns of instability and ② conveniently reproduce the peculiarities of W1327 which is probably now lost.

Rezette of W1729 Mal- \rightarrow Mal+, thus resembling W1327 original behavior.

11/21/51. Restudy ① and
plant W1729 ca = $\frac{\text{Pure Mal}+}{\text{Mal-}, \text{Mal}-}$

1. Plate Pernassay susp. EM31 Mal. ① Hold in incubator at 37.

② store 1:100 dilution in H₂O.

A Stake plate from 8 Sept 28+ or -P 36-^P No stable. ^{in up to} ~~all~~.

B Plate from ①. all susceptible either + or -P

C Plate 11/21 : 1, 2. All + or -P 1 pure + -

D 11/22 " " "

E 11/24 " " "

All 884.

3. Nos. both with a-, + colony.

W1327 x W1394.

882

November 23, 1951.

EMB Lac, 2 mol

 Mal++ > -

No Thal_v noted in platings of pooled, no Thal_v pools

November 22, 1951.

All correspondence ca. 11/51 with Umbreit & Oguristay

Steiner recalled the report (J. Bact. 1949) that S^R mutants of the Murray + Gratiá strains of *E. coli* were not improved in growth by aeration. RYS suggested that this might be a distinctive effect of *sm* on spontaneous S^R survivors (similar to *Escherichia*'s *petite* forms). Several S^R mutants of *E. coli* here were tested, and did not differ from S^S in improved growth with aeration on Lernasay, Minimal or Tryptone broth. (mut. W1177, & other K-12 S^R).

Oguristay sent two cultures labelled S and R respectively (Murray strain). These did not show NAI. of effect, but Oguristay later wrote that the early experiments were not readily reproducible.

The cultures when streaked out are highly nitrogenous, and show a majority of minute colonies that do not grow for 48 hours.

11/20 Test S1, R1 (sm. colo.). Both show AI on Lernasay.

11/21 Re-streak S1 and R1 (as received). on EMB Lac, Nutr. Ag.

#22: large colony forms apparent on EMB Lac. Practically no growth visible TSA. Reminiscent

12/16/51.

WAc 3 grown on nutrient agar bottle.

Susp. ca 2.5×10^8 /ml. Dilute to 2×10^3 /ml. UV 90 sec
plate on nutrient agar. n.g.: survival ca 500/plate!

12/28/51. Same suspension. Dilute from normal 2.5×10^8

to 10^3 /ml. 90 sec ur. Plate .2 .1 .05 ml
ca 10×35 colonies. 4?? mutants. Recheck.
None.

Plate WAc 3 on TSA + sm 100. (ca 10^8 plated; 10^2 colonies appeared)
incubate on TSIsm. Pick clean slant to WAc slant as pure and s^R
mutant

W1729 : Selectors: Crines
or non-Maltose medium.

November 24, 1951.

Streaked from EMB Mal to nutrient agar A: Mal⁺
B: Mal⁻

Note: A forms somewhat larger, rougher colonies

11/24. Picks 4 colonies from A, B. Restreak on EMB Mal and NA

11/25

A: Mal⁺ (ca 94%) and Mal⁻ in all 4 streaks

B: Mal⁻ " " " "

∴ Maltose is not immediately necessary for instability.

Incubate from nutrient agar to Pommassay, EMB Mal and NA

Difference between Mal⁺ and Mal⁻ noted again in these streakings.
(leads to mutational equilibrium).

A	205:8	B	
1	20+ 8- some ⁺ ? ca 10% +		
2	83 84+ 34-	some ⁺ , slow ⁺ 100+: 17- ⁻ V	A3 shows no + stable
3	37+ 40- 37 70+ 71- 70	37+ 40 stable ??	B3 + stable ?
	ca 100:	ca =	

A state approaching pseudoequilibrium is reached from either side.

Stable +, (-?) occur in B series.
also

Repeat with colony streaks. Streak Mal⁺ colony (A3) on EMB Mal;
= C1

6/24/52. Restreak slant of A1, B1. A1 → mostly Mal[±], and -"
B1 → all Mal⁻"

∴ no change in stability.

Stable + seems to accumulate in successive transfers, but so gradually that selector orientation pressure can't be excluded.

December 7, 1951 at seg

see 880

12/2 Cross bush 3 x 4 on minimal agar. *Prototyphlo intermedium*

12/6 Pustules from X, also parents D(0) agar.

12/9. WAc 1 heavy spor. growth

WAc 3 no "

WAc 4 faint colonial background.

WAc 3 x 4 Mostly like WAc 4. About 20 hairy spor. colonies
from thick streak.

mycelium bits, especially around mycelial fragments.

12/9. Pustules ^(spores) A, B. mycelium to minimal agar.

12/11 ++ growth, apparently pure in A, a few residual - colonies
in B. Pustules spores on minimal medium.

12/14. A. 12 colonies in thick part of streak
B. Blanks.

Pustules from 12/11 plate, A, B and from 12/14 A.
to minimal and complete. Also rising 12/9 to
"

^b
all prototyphlo.

12/16. All streaks ++

Compare 886.

1/2/52.

Repeat cross bush. Heavy residual growth of WAc 4, but spore bearing only of interests. Restreak 4 spore bearing ~~reg~~ sations 1/5/52

1/10/52 Occasional sporulated colonies finally developed. Restreaks ^(5 days!)

1/16/52 Residual growth resembling WAc 4. WAc 4 shows too much verdium to be a satisfactory mutant New growth probably at coincidence of WAc 3 - WAc 4

December 8, 1951.

- A 12/15¹. Crossed on minimal, but WAc5 ap contains >8 hours spots of +.
 B See 880 Reverse. Cross-Burnish WAc3xWAc5 on minimal agar.
12/15¹

A+B after 3-4 days show prototroph interaction, no signs of syntrophyism.

- A. 12/11 Restreak spores on minimal. 12/14. Very few colonies A, none B. (good
microculture?)

~~12/14 Restreak from 12/10 plate to minimal, complete, also replicate~~

12/15¹ ~~Restreak from 12/10 plate to minimal, complete, also replicate~~
 No colonies

(12/12-12/14. at roomtemp.).

- B. 12/12 Restreak spores on minimal agar (separate areas). Isolated 12/16. prototroph areas; background mostly auxotrophic. Repels to minimal medium.

- 12/20/51. Scant large colonies; background smaller; isolated background heavy - (auxotroph, parent) Restreak from 12/16 plating
 2: no prototrophs.

- 12/25/51. One or two prototrophs, mainly scattered. In late growth, diffuse prototrophy (formation of heterocaryons?) Restreak 1, 2 (centers of prototroph growth).

↓ complete. Repels to minimal +, - (incub. ca 30°/each).

gave sporadic prototrophy on minimal. Pseudoheterokaryosis only?
over

1/1/51. Plate 12/16/51 Sept 1st 1951
Stalks large & prototrophic or normal.

1/6/51 1 sector largely prototrophic, about 50%
"prototrophic" colonies, may be partly sectors? Restulate
some of these —
886A. 1-4. 1/10 flat growth not sponulated.

WAc 19 x x.

12/29/51.

Harvest from 4 day bottle nutrient agar.

Yield: 3×10^8 (ca 15 ml.) cell countDilute to 1.5×10^3 /ml. UV 90 sec. Plate on D(Ac)

1/1/52 Considerable variation in pigmentation. Moderate to scattered colonies (spore texture; pigment?) on ur plates. 1 yellowish colony, well on plate: stuck out & compare with col. from control plating (might be S. griseus). slight but measurable difference perceived. Nearly last dark

ca 14×10^2 colonies replicated. 11 poss mutants selected by brushing spores on D(0), D(Ac). 3 are clearcut mutants with little or no residuum. Pick from D(Ac) to sp. spore suspensions:

WAc 18, 19, 20. Start. Crossbrush these with each other and with WAc 3 on minimal agar.

WAc 3	WAc 18	WAc 18
	++	0
WAc 18		
WAc 19	++	++ past intersection
WAc 20	+	"

no residue

WAc 19	WAc 20
0	0

++ past intersection
residual?

WAc 20
0

1/10/51. Results

1/16/52

- A WAc 19 x 18 dark gum growth at intersections of smaller colonies.
- B WAc 19 x 3 Numerous, moderate sized white colonies
- C WAc 18 x 20 occasional large dark colonies against white background.

1/16/52. (room temperature :) WAc 3 x WAc 19 very heavy dark gum growth at intersections. Background very light.

WAc 18 x 20 similar, no background.
WAc 19 on this plate shows considerable background
(from intersection?)

12/31/51.

Cultures received

- a WAc 13 S. venezuelae
 b 14 S. leucostachys
 c 16 S. coerulea

15 and 17 sporulate poorly on D(0), DAc
 yellow color

Scope spores directly per. slants to $\frac{1}{2}$ ml H₂O; Estimate density by
 cyrometer. (ca 10³). Dilute to calculated 10³/ml; Treat as 883,887.

c OK.

| 1, 9. n.g. in w set (too much milling?)
 | 0 sporulated poorly on D(Ac), OK on D(0). high variability
 a. count on D(Ac) low, on D(0) o. low & same.

Re-transfer single colonies to DAc bottles for spores

WAc 16 showed two (more?) types of colonies: dark red (on DAc) and
 V. light orange red. Replic & test minimum difference. Red
colony was used for stock. lighter colonies are also, catalyly
 asporogenous. No mutants in preliminary tests.

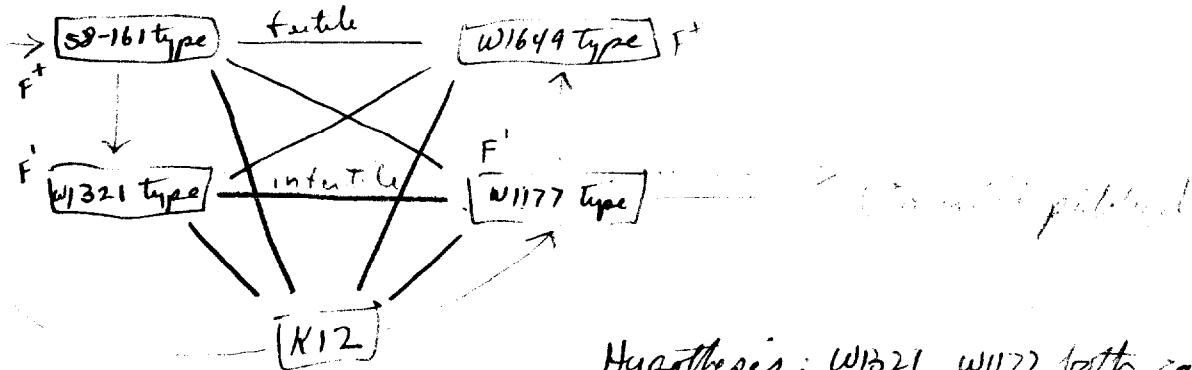
1/10/52. Re-start DAc agar bottle for spores.

See 894

Incompatibility.

1/4/58

EMF noticed that W1321 (and subline W1578 from W518) was highly infertile with W1177. Herksts and those of this experiment give following relationships:



Hypothesis: W1321, W1177 both carry the same important incompatibility allele
F⁻, all others are F⁺ and are fully self- and inter-fertile.

1/3/51. The following crosses were carried out on D(0).

F'	W1321	x		Yield
F'	W1177			0
F ⁺	W1267			++
F ⁺	W1649			++
F ⁺	W1678 (K12, pol; ser.)			+++

(58-161 x W1177 controls should have been included)
λ is significant

1/5/51. Motivate following crosses: 58-161 x W677

1. Y10 x (W1607 + 58-161) Yield 5M, Lac mostly Lac+S^d, some Lac-S^d, Lac-S^d, Lac+S^d, Lac, D(0) Repeating train D(0) to D132ec, infertile all others
 2. Y10 x W1607 + 58-161 ++ mostly Lac+S^d, some Lac-S^d, Lac-S^d, Lac+S^d
 3. Y10 x W1607 - all Lac+S^d
 4. Y10 x 58-161 ++ all Lac+S^d
 5. W1607 x (Y10 + W1649) +++ mostly Lac-S^d, some Lac+, some S^d?
 6. " x Y10 + W1649 +++ all Lac-
 7. " x Y10 - +S^d) This is evidence that F⁻ is recessive in presence of F⁺. F⁻ is then absent via "leaky" and "sterile" alleles.
 8. " x W1649 +++ all Lac-
- (Note that Lac was resistant to TTB!) +S^d)

G. pat. Ler	W1607 x	1004	++	1022	+++	W1014 x W1015	+	+
		674-680	-	1015	+++	x W1649	+	+
		477	+++	K1976	-			
		~77	-					

889: 1 8 figures of 1:200 or 0.95 Lact.

1	+, -	why are controls 2nd n.t?
2	0	
3	0	n.t. 58-161 suspension?
4	0	
5	+, -	
6	-	
7	0	
8	-	

Fertility interaction experiment apparently control'd.
Strain out exceptional photophots to list for > to use for
self-incompatibility expts.

1/7/52 W1810 x K12 (SRP) + + (Mal+ and -)
 " " x 1177 { D(0) -
 " " x 1649 } -

1/9/52 Edd. Martin to part of plate
 1/11/52 Noffert

1/10/52. Do there a connection between virage modification and F⁺?

A. 1 W1178 x W1607 : segregation of Mal, S.
 2 W588 x W1607 Lac
 3 W1178 x 58-161 Control, Seg. Mal, Lac, S. (cut. plate) 101178 sweep
 4 W677 x 58-161 " " " " apparently contains
 Lac -> Lac + Mal -> Mal + segregation of F⁺

Is λ irrelevant to F⁺/F⁻?

B. 1 W1321 x W1027 (F⁻) { n D(0). } infertile
 2 F⁻ x W1267 (F⁺) } very fertile

1 588 x 1607 Lac -> Lac +
 2 ~~617~~ x 58-161 Lac -> Lac +

3 W1800 x W1178. Mal+ > Mal- (\therefore BMF⁻ x TLB, F⁺ f, shows same
 same linkage as BMF⁺ x ...)
 see 896.

4 1178 x 58-161 Mal+ > Mal- Lac+ > Lac -
 5 1178 x W1607 Mal+ > Mal- Mal+ > Mal-

Conclusions:

1. status of W1810 unknown, check mutation
2. B shows that λ is not directly related to F
3. F⁺/F⁻ not directly related to above linkage of f. TLB,
 at least $F^+ \times F^- = F^+ \times F^+$

Conclusion: $F^+ \times F^- = F^+ \times F^+$

1/11/52 W1367 { x 679 SRP } Both fertile, segregating Lac.
 W1607 { x 679 SRP } \therefore 679 is F⁺

(see over)

W1813 x Y10 + + +

W1303 x Y10 + + +

679-183 x W1607 + +

confirms that $679 = F^+$
 $679-680 = F^-$

W1033 XX

570

Hfr.

1/6/52

Harvest 10ml Pumasay tubes to turb. "cone" xx are 1/ml each gram of peat.

D(0) plate after soil removed, add 10ml. Dil xx. one of this "

No more before plating.

- A. 1 58-161 x W1177
 2 1033 x "
 3 58-161 x W1649
 4 1033 x "
 5 58-161 x W1678
 6 1033 x "

	Cone	Dil
1	40	3 (+1?)
2	ca 70 ("rare small col")	12
3	50	0
4	17	0
5	12	0
6	31	0

- 7 Mac~~161~~ x 1033
 W118 II

0

These yields on the whole are
very low. Peat.

A. 1/151. "cone" as above. "Dil": dilute only W1033, do not alter W1177

- 1 1033 x 1177 +++
 2 " 1649 +++

cone dil.
 no effect of Hfr noted in comp. $\frac{58-161}{1033} \times$
 Re-cover Hfr!)

677

B. Aerated cultures. Resuspend in 10ml for cone. 1.1/10 for dil. Imbacto

	Cone	Dil.	D(0)
11 W1033 x 161 W1177			
12 58-161 x W1177			

~~13~~

No yield. Except for rather low yield
 generally in A. this confirms the sterility of aerated cultures. Try
 $58-161 \times W1177$ aerated non aer. various combinations to
 see if it may not act via F⁺.

1/9/52

Following cultures grown in Penassay.

1. 58-161 + W1607 + Y10 (100 ml) sediment cells and filter supernatant.
 2. 58-161 10 ml
 3. Y10 10 ml.

o No nitrate.

Add "Kornman" 1:1 Penassay. Dose. W1607 11:20 AM - 4:20 PM
 (Also dose Y10 in separate culture).

Plate o 1, 2, 3 + Y10 on D/0 1.2 ml 1607; .1 ml Y10

1/10/52 No prototrophs in any of the series 0, 1, 2, 3 or when filtrate of u 58-161 was used. F+ substance may not be present in filtrates of grown cultures.

1/10/52 Durin glass U tube, 15 ml Penassay each side.

58-161 + W1607 + Y10 vs W1607 + Y10. (A) (B)

11A14-
S30PM

Plate 6PM on D/0, EMS bac

1/11/52. A++ B- 1/12/52 A+++ mostly bac, few - B-

stimulus is not filterable!

Do stimulus inhibited?

Show $F^- S^R + F^+ S^S$ together, then inoculate into one broth to select out S^S . Test $F^- S^R$ residue from time to time.

1/9/52

Honest 58-161 from 10 ml Pem assay. Irradiate ^{11:35 - 12:00} for 20 sec. in 100 sec. UV. Incubate ^{11:35 - 12:00} in 4°C Broth 20 minutes. & dilute and cross \times W1177. (.1 ml each)

Control: 6

UV : ca 200!

This confirms Hayes' claim.

Repeat with F⁻ (F⁻, +, +) and

1/11/52. Repeat similar experiment to "activate" ^X 58-161, W1607, W1248 and W~~16~~677.

58-161 F⁺ A, A'W1607 F⁻ B etc.

W1248 X C

W677 F⁻ D

		EW1607
1	AD	++
2	A'D	+
3	A'D	±
4	A'D	+
5	B'D	++
6	B'D	+
7	B'D	+
8	C'D	+++
9	C'D	++
10	A'E	-
11	A'E	-
12	B'E	-
13	B'E	-

~~A' F⁻ to D~~ factors control factors control
~~should be all low~~ experiment
~~up to a point unless~~
~~est need~~ W1607 is activated
~~by big growth in~~
~~big. precursor~~
~~of act. precursor~~
~~not also UV < control~~

892a. 1/11/52. culture BD: effect of yeast extract incubation (as in control for UV) on 1607 F⁻.

1. W1607 \times Y102. W1607 (Y.C. Broth 20 mins) \times Y10

	D(0) ca:
11	AC 60
12	A'C 0
13	BC 100
14	B'C 10
15	A''C 30

No activation! Dose too high?
 (see over)

Controls are Y.C.
 A' UV
 A'' ~~dead~~
 saline (ctrl yeast)
 control

$$\frac{892 \text{ C}}{112/52 \pm} \quad \begin{matrix} \textcircled{1} w67 \\ \textcircled{2} w67_{uv} \end{matrix} \quad \left\{ \times w1649 \right.$$

- ② was sterile
- ③ gave high yield,

numerous lac^t heterozygotes. [Is w1649 het?]

① 4 Lac^+ \rightarrow all Lac^+ $3 \text{ Mal}^+ S^S$
 $1 \text{ Mal}^- S^R$

33 addnl lac + or? colonies picked from E 175.

16 likely bacv. Others may be tor v. 6 - .

16 likely bacv. Others may be too v. 6-
Replete single colonies. Spot on EMS bac. Bush / sm ENB Mal

3/27/52. Redbacks: w67 x w1177 highly infertile
w67 x w1876 " fertile.

∴ W67 is a very weak F+

1/10/52

Grew 58-161 A, A' in 10 ml Pernassay I aeration (& antifoam).
 W1607 B, B'
 Y10 C, C'
 W1649 D, D'

Honest treated to 5 ml
 linear to 2".

Honest and cross in following combinations; no D (0).

A-C	+++
A-D	++
A-C'	++
A-D'	+
A'-C	0
A'-D	++
A'-C'	0
A'-D'	++
B-C	0
B-C'	0
B-D	++++
B-D'	+++
B-C	0
B'-C	0
B'-D	++
B'-D'	++
B'-C'	0

- ① B-C' infertile as before
- ② A-C or A-C' futile
- ③ A-C or A-C' all crosses with D or D' were futile
- ④ A' infertile with C or C'. \therefore A' behaves like F⁻ whereas D' retains F⁺ character.

Resistance of DF⁺ to F⁻ behavior on aeration should be verified.

1/13/52 Repeat. A = 58-161 B = ~~Y10~~ C = W1649 1 = standing 2 = aerated
 $\begin{array}{l} [3 = \text{oxygenated, but } A+C \\ \text{from start mouth did not grow}] \end{array}$

A1B1	++
A1B2	++
A2B1	0
A2B2	0
A1C1	+
A1C2	+
A2C1	++
A2C2	++

This confirms previous study. 58-161 aerated behaves like F⁻. Can W1649 be inhibited by oxygen? \therefore

1/11/52

1/11 Grow W1607 with 58-161 (and separately) in Pinassay.

1/12 (A) Streak out to recover W1607 as Lac- (B) inoculate mixed culture ^{1:50}
10⁷/ml into tryptic soy broth to remove bulk of
58-161 11:30 AM - 4 PM.

C 1st cultures (before sm selection)

1	58-161	x	y10
2	1607	x	y10
3	58-161 + 1607	x	y10
4		x	y10

D(0)

EM Slac EMBS
for 58-161

+++
0
++
++

all +

D 2nd series (4 PM sm selection)

4	(58-161 + 1607)	x	y10
5	"		w677
6	1607		

++
0
6

all + -

D 3d series (9:30 2d sm selection) 4P12 - 4:30 PM.

7	58-161 + 1607	y10
8	"	w677
9	1607	y10

++
0

all + -

D 11 AM 1/13/52. 3d sm selection

10	58-161 + 1607	y10
11	1607	y10

Effect of 58-161 on W1607 seems to persist
See 896 also.

A. About 2 lac- : 1 lac+. Collect about 40-60 lac+ colonies for reisolation W1607. Mount Pinassay 1:45 PM. \rightarrow "No lac+ in streaks. No lac+ detected in streakings of 4, 7 (except 1-5 colonies $< 10^{-3}$).

II W1607 (58-161 + w1607) x y10

++

+ -

III W1607 y10.

0

C. 1/14 \uparrow Single colonies from streaks of A from Pinassay. 0 = mass culture
PM 1/14. D(0) $\frac{X}{Y} 10$

\therefore 6/7 cells were transduced
to F^+ by growth with F^+ .

Streak out on Gal EMBS to test heritability further
from single colonies

1	0
2	++
3	++
4	++
5	++
6	++
7	++
8	++
9	++
W1607	++

F' prototyphes

896

1/11/52.

1 } F' \times F - (S^S)
2 } parent of F' { 889-1
 | 889-5 of 18 Lac - 15 were S^S
 | of 13 Lac+ 4 were S^S \rightarrow 3 were Lac- after pur.
3) Prototypes from W1800 \times ~~W1178~~ W1178

4 " " #58-161 \times ~~W1177~~ ~~W1178~~ W677

SRP \times W1177 W1649

1 A	+++	+
B	+++	+
C	+++	+
D	+++	+
2 A	+++	+
B	++	+
C	++	+
D	++	+
H-12	+++	+

No F - from presumably F- \times F-
prototypes. But see 895 for
probable explanations.

January 14, 1952

c. 75 ml 58-161 harvested from Pernassay to 30 ml saline.

4 ml aliquots in each tube

2 PM - 4:30 PM 37°

	A	B x W1649
1 Refrigerate in saline	x 410	1° 11
2 Aerate " "	3 4	20 40
3 " " O ₂ (O)	1 2	20 40
4 Oxygenate " saline	0 -	60 -
5 " " O ₂ (O)	0 2	8 ✓
6 " " Pernassay (1:3)	100	40
7 Initial assay	3 5	2 4
8 Incubate in saline	0 5	1 3

Antifoam added to each tube.

Results ambiguous owing to low controls.

Repeat

ca 50 ml → ca 2 ml. [x 410]

1/16/52 1 Direct assay, 1 ml

B 2 .7 ml + 5 ml B(0)

3 " " aerate incubate.

11:50 -

+++
++

∴ Reaction of washed suspensions is influential.

1/18 Repeat aeration effect expt again.

1 58-161	x W1177	1/20
2 " "	x 899-5	++
3 58-161A	x W1177	- ✓ 1/22
4 " "	x 899-5	+++

This proves that the aeration effect is related to F (cf 3, 4 which are XX W1177, W1177F+ resp!)

1/19. 58-161 etc. grows in aerated O₂ + BM or T+B.

	A 1/20	A 21
1 58-161 x W1177	+	++ (>100)
2 58-161A x W1177 A	- ±?	5 cols
3 58-161A x W1177	- ±?	6 cols.
4 58-161A x W1817 _p A	++	++
5 58-161 x W1817 _p A	++	+
6 58-161 x W1817 _p	++	++
7 W1607 x W1177 A W1817 _p	+++	++
8 " x W1817 _p A	++	++
9 58-161 x W1177 A	++	++

Again, the aeration effect was not absolute but correlation with F+ is quite clear.

58-161A: aerated in Pernassay from 12N20-10A21
C: CO₂ bubbled from ca 6 P20 - "

E. 1 58-161	W1177	++
2 " A	W1817	±
3 " A	W1817	+++
4 " C	W1177	±
5 " C	W1817	++
6 " 4da. W1177	++	++
7 " W1817	+++	++

8. 58-161 W1304 ++ 18 cols
9. 58-161A W1304 ++ 45 "

No effect of ageing

1/24/52

Pneumay: 58161 4PM - 10 AM.

1 aerate briefly

2 N₂ bubbled briefly. Growth very poor.

Start a fresh with 1:20 dilution from ①. 11AM - 4:30 PM.

1 -

2 aerate

3 N₂4 CO₂ (7/10 NaHCO₃)N₂ still inhibits. 4:1. 2 > 1.

Harvest and cross

A W1171

B W1817

1/26/52 all plates jarred!

No apparent source of error

Plate 1/28

1 -

2 aerate ^(old) strongly3 N₂ weakly (ca 10 bubbles/minute. little growth stimulation).4 CO₂A × W1171
B × W1817

	A 1/2	B	1/30 A	B	1/30 B	1/2
1	+	-	+	++	++	++
2	-	-	-	+++	+++	++
3	++	-	++	++	++	++
4	+	delayed	+	++	++	++
5	++	delayed	++	+++	+++	+++

∴ It is air not inert gas
that causes F+ → F-. Air
does not seem to influence washed
cells, however, according to 8

Oxygenation; W1649; aeration

898

1/15/52

Glow overnight in Lumassay.

A 58-161
 B " + Air
 C " + O₂
 D W1649 ~~+~~
 E " + O₂
 F W1607
 G Y10.

In general, + = 2-20
 ++ = 20-100
 +++ = 100-600
 ++++ = > 600

1 A	D	20h.	44h.
2 B	E	+	++
3 C	G	+	++
4 D	B	++	+++
5 E	B	++	+++
6 F	G	++	+++
7 G	C	++	+++
8 H	C	++	+++
9 I	C	++	+++
10 J	F	+	+++
11 K	F	-	+++
12 L	G	-	0

No experiment

Cells recovered??

(*) was delayed.

1/16/52. Repeat test of aeration effect

A x Y10 B x W1649
 1 58-161 ++ ++
 2 58-161 aerated 3 +++

Note
 divergence of W1649 *
 with aeration!

1/17/52. A = 58-161 B = 58-161A C = W1816 D = 1816A. E = 58-161 48hrs
 F = Y10 "

1 A	Y10	++	++
2 B	"	±	5
3 C	"	+++	++
4 D	"	-	+
5 B	679-680	F+ I m	+
6 B	679-183	+ +++	++
7 B	W177	- -	8
8 B	W588	+ ++	++
9 B	W1304	+ +	+++
10 B	W1635	+ ++	++
11 B	W1649	+ ++	++
12 C	"	+	+
13 D	"	-	++
14 E	Y10	+	+
15 E	F	++	++
16 E	W1649	+	+
17 A	F	++	++
18 B	F	++ small	++

In these experiments, inhibition by aeration was not absolute. There may be recovery on the aging 36h. no effect plates themselves. The aeration, 58-161 part (b), W1816, + + + / ++ effect parallels the modification of F+ to F-, possibly excepting reaction with ~~W1304~~ W1304 which should be checked in a controlled experiment. Again note possible greater purity of F+ x F- vs. F+ x F+

1/15/52

1. Washed cells. Mix 58-161 and W-1607 in saline. Incubate 3 - 9 PM. a) Streak out on EMB Lac; b) Inoc. Penassay + sm 10ug/ml. c: λ^+ All XX x Y-10 unless indicated
2. Grow 58-161 + W1321 in Penassay. a&b as above. (a showed background of Lac+ and lambda plaques. λ λ^+ (therefore λ has not been transmitted unless F^+ has). lysogenic
3. Transmission from lambda-sensitive to ~~lambda~~. W-1655 + W-1607 in Penassay. Then a&b c: λ^+
4. c: λ^+ to sensitive. W-1655 + W-1321....
5. F^+ to W-1177. W-588 + W-1177 a & b. (b showed rare + pap. b culture was pure.)

In all cases, Lac- colonies from a) were pooled to make fresh inoculum. Cultures were restreaked to control success of reisolation of F- component. Growth from sm-Penassay in b) was used directly, in each case with only barely detectable Lac+ residuum.

6. Transfer via lambda? 58-161 streaked out on W-1321 on EMB Lac sm. Plaques restreaked. Individual colonies picked and tested for lysogenicity. #1 (out of ca 25) was lysogenic and restreaked, rechecked. Singh verified lysogenic colony retained for test of F^+ (X y-10)
7. Single colonies of W-1816 from stock culture (itself reisolated) streaked on EMB Gal(-). o = stock.

Tests (x Y-10 exc. 5, x W-1607)

	a	b	c (= retest pooled Lac- colonies from test streaks of b)
1	-	7 col. + ?	w1607 cont: -
2 ++	++	++	
3 +	+++	+++	
4 ++	++	+++	
5		+++ C: 1+++ 3+++ 5+++ 7+++	w1177 cont - 4 = W1817 (W1177F+)
6	-	.	.

$\therefore \lambda$ does not transmit F^+ . Cf. 2.

7 1:+++ 2:+++ 3:+++ 4:+++ 0:+++ W-1607 -. F^+ of W-1816 is therefore heritable at least 50 cell generations.

8. Crude supernatant of 58-161. Add sm 10ug/ml. Inoc. W-1607, incubate 1-7 PM. Limited overall growth! Reinoculate loopful to Penassay sm for cross inoculum, and streak out on EMB Lac. (ca 1% Lac+)

9. Whole culture 58-161 inoc. ca 1:20 sm Penassay. Add W-1607 as above.... < .1% Lac+

10. Dense cell suspension 58-161 aerated, as above.... < .1% Lac+
Crosses of sm-selected W-1607 treated component.

8 ++ Is this due to the residual cells? Should be plated out without λ growth cycle.
9 +++
10 +++

Washed cell mixtures are inefficient in transferring F^+ . λ and F^+ are distinct.

Persistence of ad factors effects on F+

900

January 17, 1952.

From various expts. streak out aerated cultures that have shown partial or complete inhibition of F+. Pick single colonies to 1 ml Pernassay and test for F (\times y10, w1177 or w1817) by "unashed" crosses.

- A. 898C - B (58-161) 5 cols }
 B. 898C - D (w1816) 5 cols. } all F+
- C 897 1/18 58-161 5 cols. F+ ~~F+~~ F+
- D 897D 58-161 synth. 5 cols. F+
- E 897D 58-161 A 1/20 \rightarrow 1/21 11AM \downarrow 4: 1F+ 2F- 1F±? E3F+ E1F-
 F " " C. 4: F+

G Remov. E for iterated aeration 1/21, 2:30 PM \rightarrow 2F+ 2F- : G1F- ✓ G2F- ✓

H " \downarrow 1/22 11AM 3F+ 1-? (eastern. in test plate)

I " \downarrow 1/22 5PM 2F+ 2- ? "

J " \downarrow 1/22 830 PM ? east? F- "

K " \downarrow 1/23 900 AM F+ (Knospemaria \times w1177 was +)

L " Same E $\overset{= \text{W1830}}{\text{I}}$ for further tests.

M " $\overset{\text{later tested TLB, -}}{\text{a) Re-transduction to F+}}$

N " $\overset{4.9}{\text{b) comp. xx w1177, w1817 : by first test, slightly}}$
 in all combinations!

LA Fast growth for F-? (see 58-161 to aer. Pernassay ca 1:100 1215
 B streaks out 1215
 C 315 min. } held in refrigerator

Contamination
or culture transport?

No persistent F- found in this series!